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Opalina mitotica.

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With plate 2 and 1 figure in the text.

In this very interesting species the nuclei rest in a mid-mitotic condition instead of in the network or granular condition as in all other known animals and plants. These Opalinas were discovered by Professor J. H. Powers of the University of Nebraska. He has sent me a half-dozen beautifuly prepared slides of these parasites and asks me to describe them, making only the condition that the species be not named for him.

In answer to my questions Professor Powers' daughter, Miss H. H. Powers, writes: „They (the Opalinas) were taken from the rectum and large intestine of *Amblystoma tigrinum* late in the fall. The specimens from which they were obtained were all young animals that been fed in water for a month or more on chopped liver. During the fall a few of the animals so kept usually die unless taken from the water at this their period of hibernation. They seem morely to get too lazy to rise to the surface for air. It was from such animals as these, taken a few hours after death, that the Opalinas were obtained by removing the digestive canal and opening it in normal salt solution. They were washed out in this solution together with the contents of the rectum. In this normal salt solution the Opalinas were gradually isolated by tilting and shaking the dish in various ways until they could be drawn off with a pipette and placed in the killing fluid.

"The time from opening the animal to the killing of the Opalinas varied from a few minutes to about four hours. This made little, if any, difference with the nuclei. Those examined fresh showed apparently the same condition. It is possible that the nucleus rests in this mitotic condition, or possibly the death of the host may have induced mitosis. We have been unable to determine this point for lack of material. Our older living salamanders do not seem to contain many living Opalinas, and the young animals, which are usually so abundant here in the west, have been unobtainable during the last two years."

"The killing fluid used was one that we can recommend highly for protozoan nuclei, namely a mixture of equal parts of KLEINENBERG's picro-sulphuric acid (strong formula) and concentrated sublimate in five per cent acetic acid. The stains were CONKLIN's picro-haematoxylin, used very weak and prolonged several days, or else different carmine stains followed by combinations of Lyons blue and picric acid in absolute alcohol. The clearing was done with different grades and combinations of Bergamot oil and Xylol, followed by gum damar introduced in the lump."

"We have not found these Opalinas in any other of the several species of Amphibia which we have examined here."

Only three points in this letter call for comment. The presence of the Opalinas in the large intestine, as well as in the rectum, was probably due to the hosts having died some hours previous to the removal of the Opalinas. In living frogs, if in good health, the parasitic Opalinas are restricted to the rectum. Often after the death of the host some of the parasites pass into the intestine. In one instance, in a frog with badly inflamed stomach and intestine, I have found Opalinas in the intestine while the frog was still alive. Probably in the living *Ablystoma* the Opalinas are confined to the rectum.

The suggestion that mitosis in these Opalinas may have been induced by the death of the host is a natural one, but is very improbable, for in other species of Opalina, though abnormal conditions, such as cultivation outside the host, tends to bring on mitosis, this change in the nuclei appears only after one or more days and never in many individuals, whereas the Opalinas here described all show nuclei in mitosis. It seems clear that the nuclei rest in a mid-mitotic condition.

The fact that these Opalinas are more abundant in young salamanders taken in the fall than in adults — presumably examined in the spring when they are more easily captured — is probably due to the porasites leaving the recta of the adult salamanders in the spring when they go to the water to breed.

In its general form *Opalina mitotica* resembles *O. intestinalis* (Figs. 1—6, 8 and 10), being a spindle pointed posteriorly and rounded anteriorly and with its anterior end bent to one side, as in the case with all known *Opalinae*. The anterior rounded end is wedge-shaped, to that in edge view it looks almost sharp instead of broadly rounded (Figs. 3 and 4). In this regard this species resembles *Opalina zelleri* and the little known *O. flava*. The relative general proportions are about as in *Opalina intestinalis*. The size of the specimens examined varied from a length of 94 mm and a greatest breadth of 23 mm in the largest individuals to a length of 52 mm and a with of 9 mm in the smallest.[1]) Of course in the spring much smaller individuals would be found.

There is generally a somewhat greater distance between the nuclei than in *Opalina intestinalis*. The two nuclei when fully formed are never connected by a strand as is so often the case in *O. intestinalis* and *O. caudata*.

In size and number of the plastids *O. mitotica* agress with *O. intestinalis*. The endosarc plastids are disc-shaped, the flat sides of the disc being parallel with the surface of body (Figs. 14 and 15). Upon focussing near the edge of the animal the discs, in edge view, appear linear. In the character of the ectosarc and endosarc *O. mitotica* and *O. intestinalis* closely agree. In individuals stained with carmine and picric acid the endosarc is red and the ectosarc green. The ectosarc plastids are large and irregular and stain differently from the endosarc plastids.

None of the preparations I have show an excretory vacuole, but in a few individuals there is an axial region which looks exactly as does the axial region of an *Opalina intestinalis* with a well developed axial vacuole, which has been killed in corosive sublimate-acetic acid and stained in Delafield's haematoxylin (cf. Metcalf 1907). It seems therefore that *O. mitotica* has a well developed axial vacuole, but study of living individuals is needed to confirm this point with certainty. If a well developed excretory vacuole is present in this species, cursory exmmination of living individuals which have been kept a few hours in normal salt solution would doubtless show a mass of small coccus-like granules entangled in the posterior cilia or dragging in an irregular strand behind the animals as they swim. The well developed excretory vacuole of *O. intestinalis*, *O. caudata*, or of an undescribed species I am now studying, occasionally contracts, extruding granules from the excretory pore at the posterior end of the body. Probably all cylindrical species of Opalina have well developed vacuoles. In the flattened species the vacuoles are reduced or absent. None of the specimens I have show any special mass of granules among the cilia at the posterior end of the body, but preserved specimens of *O. intestinalis* or *O. caudata* almost never show the extruded granules.

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[1]) Excepting two individuals just from division.

The cilia show no unusual features.

It is in the nuclei of this Opalina that the phenomena of chief interest are seen. *Opalina mitotica* is a binucleated form, but each of its nuclei, under ordinary conditions, is dumbbell-shaped and is seen to be resting in a late anaphase of mitosis (Figs. 1—5 and 14). None of my specimens show the mitotic phenomena as clearly as could be desired. The nuclei stained with carmine are not at all clear even when examined by green light, and those stained in CONKLIN's picro-haematoxylin do not give nearly as good results as are obtained in *Opalina intestinalis* and *O. caudata* by heavily overstaining with DELAFIELD's haematoxylin and decolorizing rather rapidly under the microscope. Counts of the chromatin masses (chromosomes) have been made for one or both nuclei in about twenty-five of the more favorable individuals. The number seems to be ten, though none of the nuclei show their structure with the absolute clearness desirable for counting such irregularly shaped chromosomes (Figs. 7, 9 and 14). I have however no doubt of the correctness of the counts.

The irregular form of the chromosomes reminds one of conditions in *O. intestinalis* and *O. caudata*, as does also the character of the fibres of the mitotic spindle and the appearance of the whole mitosis (Fig. 9). In one nucleus there was found a fusion of the chromosomes into a ring (Fig. 13), as is so characteristic of the late anaphase stage of mitosis in *O. intestinalis* and *O. caudata*, especially if these animals have been kept for a few days outside the host in normal salt solution. The same phenomena of fusion of the chromosomes is often seen in nuclei of freshly taken Opalinas of the two species mentioned. It seems to be a normal phenomenon which is emphasized under abnormal conditions. In *O. saturnalis*, as described by LEGER and DUBOSC, the chromosomes at one stage form a ring around the nucleus. These authors do not say if the ring formation is as frequent and as marked in freshly taken individuals.

In *Opalina mitotica*, as in all other *Opalinae* studied, the nuclear membrane persists, not disappearing at any stage of mitosis.

None of the material studied shows the formation of chromatin spherules from the chromosomes, as I have described for *O. intestinalis* and *O. caudata*. This is probably due to the methods of preparation. The nuclei, being in a late anaphase, are past the stage in mitosis when the chromatin spherules are most distinct. Most should by this time be disselved, but often in other species one finds a few chromatin spherules that for some reason persist a little longer, even as late as the anaphases. None of the nuclei in my specimens are sufficiently deeply stained to bring out these bodies if present.

No nucleolus appears in any nucleus in my specimens. This may very probably be due to the methods of preparation, for in *O. intestinalis* and *O. caudata* the nucleolus is not brought out by many of the common fixing fluids and stains. Some careful students have denied its presence in those species, though some methods demonstrate it with great clearness and though Zeller had accurately described it in 1877. "Lichtgreen" after sublimate-acetic brings it out very clearly, so does acetic acid alone (Zeller's method). In *O. intestinalis* and *O. caudata* the nucleolus persists throughout the mitosis, going undivided into one of the daughter nuclei, the other daughter nucleus acquiring a new nucleolus. It is therefore improbable that the nucleoli in these specimens are absent because they have disappeared during mitosis, since in other species the nucleolus does not so disappear.

Both longitudinal and transverse division occurs in *Opalina mitotica*. None of the eight hundred and thirthy-three, individuals in my slides show the body really in process of division, but there is one which is slightly constricted in the middle, as if beginning to divide transversely, and another which is broadened and furrowed preparatory for longitudinal division, and there are present in the slides twenty or more individuals that have just been formed by longitudinal division (Fig. 6), and there is at least one individual 1)

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1) There are in the slides two other individuals which are very short and stocky and seem to have been recently formed by transverse division. Both have recovered from the division and show an even contour and complete ciliation (Fig. 10).

which is the posterior of a pair recently formed by transverse division (Fig. 11). Longitudinal division seems, therefore, more common than transverse division, as is true for other species of Opalina. Opalina has both the Flagellate type of division (longitudinal) and the Ciliate type (transverse), but the former is much more frequent. Probably this is an archaic character.

One of my specimens shows an interesting condition of the nucleus (Figs. 8 and 9). It is an individual which has just come from longitudinal division. Its only nucleus is seen to be almost divided into two, and one observes that each daughter nucleus is already in the somewhat diffuse equatorial plate stage of mitosis preparatory to establishing the condition characteristic of fully formed resting individuals, i. e. the condition with two nuclei each dumbbell-shaped and in a late anaphase of mitosis. The mitotic figure resembles closely that seen in *O. intestinalis* and *O. caudata* at a similar stage of nuclear division.

The structural conditions in *O. mitotica*, as described above, suggest discussion of the nuclear conditions in general in the *Opalinae* and in the *Ciliata* as a whole. Heretofore there have been known four species of binucleate Opalinas — *saturnalis* (LEGER-DUBOSC), *intestinalis* (STEIN), *caudata* (ZELLER) and *macronucleata* (BEZZENBERGER). RAFF has just described a fifth — *O. hylarum* — and a doubtful sixth — *O. binucleata* — which I suspect may prove to be but an unusually flattened form of *O. macronucleata*. I have found in *Bufo agua*, from Jamaica, a very similar form which I am still studying. To these four to six binucleate species must now be added the very distinct *O. mitotica*. One quadrinucleate species is known — *lanceolata* (BEZZENBERGER). Eight multinucleate forms have been described — *dimidiata* (STEIN), *longa* (BEZZENBERGER), *flava* (STOKES), *lata* (BEZZENBERGER), *ranarum* (EHRENBERG), *coracoidea* (BEZZENBERGER), and *obtrigona* (STEIN). DOBELL will soon describe a ninth to be named *O. virgula*, and I shall soon describe a tenth, calling it *O. intermedia*. I will postpone till a later paper a discussion of the remarkable intergradations between the multinucleate species.

Opalina mitotica is especially interesting as showing a transition from the binucleate species like *intestinalis* and *caudata*, on the one hand, and the quadrinucleate *lanceolata*, on the other hand. In *intestinalis* the two nuclei are “resting nuclei”, with their chromatin in the typical network condition. *O. mitotica* has each of its two nuclei half divided and resting in this mid-mitotic condition, each nucleus being dumbbell-shaped and with its chromatin in a late anaphase of mitosis. A very slight advance in nuclear division before coming to rest would give the quadrinucleate condition, as in *O. lanceolata.*

In a previous paper I have expressed the opinion that the binucleate condition of the four binucleate species then known was due to a delay in the division of the body,[1]) the nuclei dividing and the cell-body remaining for a long time undivided, its division occurring later just as the two nuclei are preparing to enter upon their next division. In *O. mitotica* the two nuclei actually enter upon this second division and almost complete it before coming to rest, and the cell body still remains undivided. Its division takes place just as the second nuclear division is completed, not when it is beginning as in *O. intestinalis.* In *O. lanceolata* the division of the body is still further delayed. The two nuclei not only enter upon but complete the second division, until four nuclei are formed, and still the body remains undivided. The division of the body does not occur until these four nuclei enter upon the third division. The quadrinucleate condition is thus characteristic of this species. The multinucleate condition of other species seems to be due to a still further delay in the division of the body, while nuclear division continues, resulting in many nuclei within one body. To be sure the delayed divisions are merely delayed, not entirely suppressed, for, in the spring, division of the body takes place rapidly, more rapidly than division of the nuclei, until finally uninuclear gametes are formed.

The Opalinas are only temporarily binucleate. The higher *Ciliata* have become permantly binucleate by the complete suppression of one division of the cell body. The permanent binucleate condition of the higher *Ciliata* allows differentiation between the nuclei, one being active in nutrition (the macronucleus), the other (the micronucleus) remaining comparatively inactive chemically, but awaking to activity when division occurs. The micronucleus is the sole carrier of heredity, for the macronucleus takes no share in the sexual phenomena, but degenerates. It is evidently easier to form

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1) "It seems probable that the Opalinas were originally uninucleated, that the binucleated condition was brought about by the suppression of one division of the body when the nucleus divided, and that the multinucleated condition is still more secondary, being due to further suppression of divisions of the body" (Metcalf, 1909, p. 308).

a new macronucleus by metamorphosis of a micronucleus than to carry the highly specialized and hypertrophied macronucleus through the stages of conjugation. On the other hand in the Opalinas the transient character of the binucleate condition does not allow any such divergence and specialization in the nuclei. The Opalinas have not developed micronuclei and macronuclei, but each nucleus is an ordinary unspecialized nucleus. In a previous paper I have written upon this subject as follows (METCALF, 1909, p. 272): "The question as to what in Opalina is the full homolog of the macronucleus in higher *Ciliata* can best be approached through a discussion of the evolution of the condition with two functionally diverse nuclei. The macronucleus of higher *Ciliata* arises by the metamorphosis of a nucleus which has itself arisen by division from the micronucleus. It is therefore phylogenetically a complete nucleus and not a mere mass of granules extruded from a nucleus and gathered into a group. The macronucleus seems to be specialized in connection with the nutrition of the cell. It is able to divide, as does the micronucleus, in the vegetative divisions of the cell, but it takes no part in the special phenomena, interpreted as maturation, which precede conjugation. The micronucleus apparently holds in abeyance the functions connected with the nutrition of the cell, but the potentiality of these functions must be present, since daughter nuclei from the micronuclei are able to transform into macronuclei. Probably each type of nucleus is a complete nucleus, the nucleus especially connected with conjugation being slightly specialized by diminution of some of its functions and probably of the chromatic material upon which these functions rest. The the macronucleus is specialized by the great development of its nutritive activities and a corresponding great increase in the amount of chromatin especially associated with these functions. The specialization and hypertrophy of the macronucleus seems to have gone so far that it is difficult to secure conjugation of the macronuclei, and so, partly as a consequence, the macronuclei degenerate. The germinal (unspecialized) chromatin is so overbalanced by nutritive (specialized) chromatin in the macronuclei that it is unable to assert itself and bring about conjugation of these nuclei, and without occasional readjustment such as is secured through conjugation, ultimate degeneration seems unavoidable.

"Before copulation or conjugation there seems to be quite generally, among the Protozoa at least, a process of reestablishing the proper balance of the nutritive and other chromatin in the nucleus.

It is apparently the nutritive chromatin which especially increases in amount during growth and ordinary vegetative divisions and the excess of this nutritive chromatin is gotten rid of before conjugation by the formation of chromidia, either the excess of vegetative chromatin leaving the nucleus, or the excess of this specialized chromatin being left in the nucleus, the ordinary chromatin going out into the cytoplasm and there reformig into a new nucleus or new nuclei, or, as in *Chromidina* (Gonder 1905), all the chromatin passing into the cytoplasm where after a time a part degenerates and the rest forms the generative nuclei. We doubtless do not know the full significance of these phenomena, but this much seems probable, that there is division of labor between different parts of the chromatin and consequent hypertrophy of some parts during the periods of special activity. The specialization and hypertrophy of chromatin in connection with nutrition has gone so far in the macronucleus of *Ciliata* that it is simpler to secure a new macronucleus than to reestablish in the old macronucleus such a balance of the respective parts as will allow it to share in conjugation.

"What was the phylogenetic origin of the condition with two nuclei, one of which is highly developed for nutrition while the other remains minute and hardly shares in the activities of growth. The divergence must have occured in a binucleated (or multinucleated) condition. We have in Opalina such a binucleated (or multinucleated) form. In what way could its condition with similar nuclei be changed into a condition with dissimilar nuclei?

"First let us note again the fact that the nuclei of the binucleated *Opalinae* are often slightly dissimilar in regard to mitosis, one being often in a slightly more advanced condition than the other. There is a similar divergence in regard to the formation of the probably nutritive chromatin spherules, one nucleus showing these in a more advanced stage of formation. The exact balance of the two nuclei seems already somewhat disturbed in Opalina.

"May we conceive this divergence as going further, the nutritive chromatin becoming hypertrophied in one nucleus and not in the other, the second nucleus ultimately giving up almost all its connection with nutrition and becoming much smaller, giving us ultimately the condition seen in higher *Ciliata* with very divergent micro- and macronuclei?

"One thing seems to stand in the way of such an interpretation so far as Opalina is concerned: in the division of Opalina one whole nucleus, and not two half nuclei, is given to each daughter cell. The condition in Opalina is not a true binucleated condition. We have merely a delayed division of the body, which causes two daughter nuclei to lie for a long time in one cell, indeed even until they have entered upon the next mitosis. Division of the cell, when it does occur, is not associated with the mitosis in the nuclei which is taking place at the same time, but is really the delayed cell-division that belongs with the last nuclear mitosis. Division of the cell-body lags one step behind the division of the nuclei. To get a proper understanding of the real meaning of this division we must bring together that division of the body and that division of the nucleus which really belong together.

"In attempting to do this we see at once that the direction of the division of either nucleus or body must be changed. At present the long axes of nuclei and body coincide and remain constantly in this relation. The nucleus divides transversely and the body generally longitudinally. Can we find a plausible scheme which will get around this difficulty?

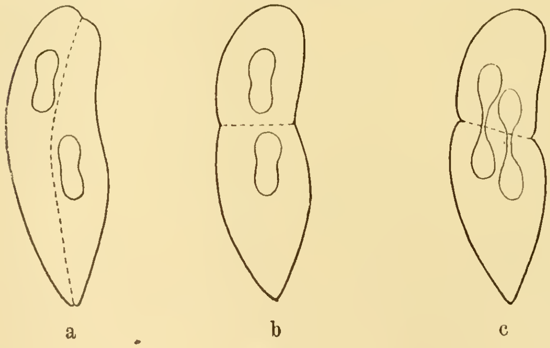

Fig. A.

"The present condition in Opalina, with an apparent but not a true binucleated state, could be changed into a true binucleated state comparable to that of Paramoecium, if cell-division should change from longitudinal to transverse and at the same time should bisect each of the two nuclei. Instead of the condition shown in Text Fig. IX. A (here copied in Fig. A, a), as now, we would have that illustrated in Text Fig. IX, C (here Fig. A, c) each cell receiving two daughter nuclei instead of one whole nucleus. From this condition, that of Paramoecium could be reached by functional and accompanying structural divergence of the nuclei, as suggested above. The ordinary infrequent transverse divisions of the binucleated *Opalinae* do not help us in this schema, for they do not bisect the nuclei (Text Fig. IX, B, here Fig. A, b). The false binucleated condition of Opalina can be changed to a real binucleated condition only by the complete suppression, not the mere delay, of one division of the body. Were this to occur, then a transverse division of the body, such as we occasionally find, would bisect the two nuclei, being not a delayed division belonging to the last mitosis, but the division which properly belongs with the present mitosis of the two nuclei (Fig. A, c). We can conceive the same result as following still longer delay in the postponed division of the body, it not occuring until the two daughter nuclei are separated to a considerable distance, so that the division of the body (transverse in this case) could easily pass between the daughter nuclei, producing thus in each daughter cell a truly binucleated condition.

"It seems to me quite probable that such has been the history of the evolution of the binucleated condition in higher *Ciliata:* first delay in division of the body, establishing a temporary binucleated condition; then complete suppression of this delayed division of the cell-body, establishing a true binucleated condition, each nucleus, as apparently now in Paramoecium, belonging to a potentially, but not actually, independent individual.

"The truly binucleated forms, as well as the falsely binucleated *Opalinae*, are really potentially double individuals; and similarly the multinucleated *Opalinae*, arising by further temporary suppression of divisions of the body, are highly compound forms composed of many potential individuals. These individuals all become ultimately distinct before or in connection with copulation, even in the multinucleated *Opalinae*, the temporarily suppressed divisions of the body finally appearing rapidly in the spring and producing unicellular gametes."

The tendency to assume a multinucleate condition is not restricted to the Opalinas alone among the *Ciliata*; there are several species which, at least under certain conditions, are multinucleate, e. g. *Loxodes rostrum* and the form recently described by Power's and Mitchell, *Paramoecium multimicronucleata.*

The most striking peculiarity in *O. mitotica* is the one from which I have named the species, namely the fact that its nuclei "rest" in the midst of mitosis, their chromatin showing a late anaphase condition and not forming a network or being in scattered granules. I know of no other animal or plant of which this is true. Why should all other nuclei "rest" with their chromatin in a granular or network condition? Why should mitosis in all other species, when entered upon, be promptly completed? Is it merely that the more diffuse granular or netted condition of chromatin enables it better to perform its chemical functions? We know from MEVES' work upon renal cells of salamanders that secretory activity seems to be lessened during mitosis. Possibly the more compact condition of the chromatin when gathered into chromosomes during mitosis is less advantageous for the chemical activity of the chromatin. But if this is the whole reason for all other nuclei hastening to complete the mitotic phenomena when once they are begun, it is difficult to see why *Opalina mitotica* alone, of all known animals and plants, should refuse this advantage and permit its nuclei to rest in mid-mitosis. The fact that it uses predigested food and so its nuclei are less active in enzyme formation is not a sufficient explanation, for this is equally true of many other intestinal parasites whose mitosis does not remain incomplete.

Growth is occurring in these Opalinas with anaphase nuclei for in individuals of all sizes the same condition of the nuclei is seen. The nuclei must be chemically active.

Is there any regard in which the nuclei of Opalina are peculiar, and if so can any suggestion be obtained from this peculiarity, that may throw light on our question? As described in a previous paper (METCALF 1909), the whole mitosis of Opalina is peculiar. There is in the Opalinas no trace of centrosome or of definite achromatic spindle fibres. The irregular mitotic spindle in this genus is formed by strands of chromatin, as it were linear, branching pseudopodia from the chromosomes. The migration of the chromosomes to the poles seems to be due partly to the thickening and consequent shortening of the chromatin spindle fibres which run from the chromatin to their attachment to the nuclear membrane at the poles of the nucleus (l. c.: "this membrane, we remember, persists, not dissappearing at any stage of mitosis"), and partly to an absorption of these spindle fibres into the chromosomes, the chromosomes absorbing the spindle fibres as they crawl along these fibres toward the poles. There is no mechanical need of any centrosome to serve as a point of resistence or attachment for the spindle fibres, for the very tough nuclear membrane serves this purpose. The daughter chromosomes merely crawl apart from one another.

Is it anything connected with this absence of centrosomes and achromatic spindle which may explain the delay in completing the mitotic process? Alveolar achromatic material is present in the nucleus of Opalina, occupying the whole inner portion of the nucleus, the chromatin reticulum lying just beneath the nuclear membrane. Could the lack of arrangement of this achromatic material in a spindle, and the lack of a centrosome, allow the failure promptly to complete mitosis? Among plants the phanerogams have no centrosomes, but they do have achromatic spindle fibres. The achromatic part of the mitotic figure is not wanting in them. In Opalina the achromatic material does not assume any specially marked form in connection with mitosis. Can it be this lack that allows the nucleus to "rest" before completing its mitosis?

In the mitosis of other genera the achromatic material is prominent. In the mitosis of Opalina it has a diminished, apparently a very slight, role, if any. The chromatin seems to be the active element. Mitosis in full sized *Opalina intestinalis* and *O. caudata* is very slow, occupying from a few hours to several days. Yet in these species it becomes complete before the nuclei come to rest. The chromatin of the Opalinas seems to be under no compulsion from centrosomes or other achromatic source. It is ordinarily very deliberate in its self-directed mitosis. In *O. mitotica* it chooses to come to rest before completing the mitosis and there is nothing to say it nay. There seems to be some tendency toward suppression of divisions of the body and toward promotion of division of nuclei. This added relative emphasis upon nuclear division is sufficient in *O. mitotica* to cause the nuclei to enter upon division, but it is not enough to carry the division promptly to completion. I see no way of finding any mechanical explanation of the failure promtly to complete the mitosis, nor do I see any necessity for seeking such a mechanical explanation. Might we not come nearer the truth if we should say that the chromatin does not choose to complete the mitosis? The further question why it does not so choose seems beyond our ken. One cannot but doubt if the mitosis would remain incomplete, even in one species of this genus, were the mitotic figure a fully developed one. It may be the imperfection of the achromatic part of the mitotic figure, throwing all the activity upon the chromatin, that allows this departure from the otherwise universal behavior in mitosis.

I desire to express to Professor Powers, the discoverer of this species, my heartiest thanks for the privilege of describing and discussing it.

Oberlin, Ohio, U. S. A., May 15th, 1911.

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Explanation of the figures.

All figures are of *Opalina mitotica.* Figures 1—6, 8, 10 and 11 are magnified 270 diameters: figures 7, 9, 12—15 are magnified 1114 diameters.

Plate 2.

Figs. 1—5. Full sized binucleate individuals of the usual sort.

Fig. 6. An individual just formed by longitudinal division. The irregular contour on one side of the posterior half of the body is the usual distortion caused by longitudinal division, the two individuals of the pair pulling apart usually with considerable difficulty.

Fig. 7. A more enlarged drawing of the nucleus of the individual shown in Fig. 6. The nucleus is in the typical "resting condition" for this species i. e. is dumbbell-shaped and in the anaphase of mitosis. Ten chromosomes appear in one end, the other end bears eleven chromatin bodies, but two are connected and probably represent one chromosome.

Fig. 8. An individual recently formed by longitudinal division.

Fig. 9. A more enlarged drawing of the nucleus of the individual shown in Fig. 8. The nucleus has begun to divide preparatory to producing a typical binucleate individual. Each half of the dumbbell-shaped nucleus is in the irregular equatorial plate stage of mitosis characteristic of the binucleate Opalinas. By the time the two halves of the dumbbell-shaped nucleus have completely separated, loosing their connecting thread, each portion will itself have assumed a dumbbell form and will be resting in the typical anaphase, as seen in Fig. 7. Ten chromosomes were seen in one end of the nucleus. Only eight could be made out in the other end, the left portion of this end of the nucleus not being clear.

Fig. 10. A small individual which has just been formed probably by transverse division. It contains the anterior of the parent nuclei. This

has not yet begun to divide, but is still in the peculiar resting condition characteristic of this species.

Fig. 11. A small Opalina just formed by transverse division. It is the posterior one of the pair. Its anterior edge has not yet restored its even contour, nor have cilia yet appeared upon this portion of the body.

Fig. 12. Endosarc plastids: a) two plastids in side view; b) two plastids in edge view.

Fig. 13. A nucleus in which one sees the chromosomes in one end united to form a continuous band. This condition is often seen in other binucleate Opalinas in slightly abnormal nuclei and in some nuclei that appear to be normal.

Fig. 14. A broken individual one-half of whose nucleus projects from the body and is unusually clearly seen. Ten chromosomes were present in the exposed end of the nucleus. In the other end they could not be counted.

Fig. 15. A portion of the middle of the body, showing the endosarc plastids. Observe that the plastids in the middle, seen in side view, appear round or broadly oval, while those near the sides of the body, seen in edge view, are elongated. The plastids must therefore be disc-shaped, with their broad sides parallel to the surface of the body. In the ectosarc are seen large spaces. These contain irregular ectosarc plastids which are not drawn. The cilia with their basal granules are indicated.